

**Amendments to the Claims:**

This listing of claims will replace, without prejudice, all prior versions, and listings, of claim in this application.

**Listing of Claims:**

1.       (*Original*) A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:
  - a) combining a macrolide starting material, a polar solvent, a hydrocarbon solvent, and water, whereby at least two phases are formed, at least one of which is a water-rich phase, and wherein the pH of the water-rich phase is at least about 7,
  - b) maintaining the combination at for at least 1 hour, whereby a macrolide-rich phase is formed from which the macrolide crystallizes.
2.       (*Original*) The method of claim 1 further comprising the step of isolating the macrolide that crystallizes.
3.       (*Original*) The method of claim 1 wherein the combination of step b is maintained at a temperature of from about -15°C to about 50°C.
4.       (*Original*) The method of claim 3 wherein the combination of step b is maintained at a temperature of from about -5°C to about 40°C.
5.       (*Original*) The method of claim 4 wherein the combination of step b is maintained at a temperature of from about -2°C and about 35°C.
6.       (*Original*) The method of claim 1 wherein the combination of step b is maintained for between 48 and 100 hours.

7. (Original) The method of claim 1 wherein the polar solvent is selected from the group consisting of alcohols, esters, nitriles and ethers.
8. (Original) The method of claim 7 wherein the polar solvent is selected from the group consisting of ethyl acetate, acetonitrile, methanol, ethanol, *n*-propanol, *iso*-propanol, *n*-butanol, *iso*-butanol, acetone, diisopropyl ether, dimethyl formamide, and dimethyl acetamide.
9. (Original) The method of claim 8 wherein the polar solvent is ethyl acetate.
10. (Original) The method of claim 1 wherein the hydrocarbon solvent is selected from the group consisting of *n*-hexane, *n*-heptane, octane, *iso*-octane, cyclohexane, methylcyclohexane, benzene, toluene, and xylene.
11. (Original) The method of claim 10 wherein the hydrocarbon solvent is *n*-hexane.
12. (Original) The method of claim 1 wherein the pH of the water-rich phase is about 8 or higher.
13. (Original) The method of claim 1 wherein the water comprises a base selected from NaOH, KOH, Ca(OH)<sub>2</sub>, NH<sub>3</sub>, Et<sub>3</sub>N, diethylamine and pyridine.
14. (Currently Amended) The method of claim 1 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, ~~and~~ everolimus, and ascomycin.
15. (Original) A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:
  - a) combining a concentrate residue from whole-broth extraction of macrolide-containing biomatter in a polar solvent with a hydrocarbon solvent, and water, whereby at

least two phases are formed, at least one of which is a water-rich phase, and wherein the pH of the water-rich phase is at least about 7,

b) maintaining the combination at for at least 1 hour, whereby a macrolide-rich phase is formed from which the macrolide crystallizes.

16. (Original) The method of claim 15 further comprising the step of isolating the macrolide that crystallizes.

17. (Original) The method of claim 15 wherein the combination of step b is maintained at a temperature of from about -15°C to about 50°C.

18. (Original) The method of claim 17 wherein the combination of step b is maintained at a temperature of from about -5°C to about 40°C.

19. (Original) The method of claim 18 wherein the combination of step b is maintained at a temperature of from about -2°C and about 35°C.

20. (Currently amended) The method of claim 15 wherein the combination of step b is maintained for between 48 and 100 hours.

21. (Original) The method of claim 15 wherein the polar solvent is selected from the group consisting of alcohols, esters, nitriles and ethers.

22. (Original) The method of claim 21 wherein the polar solvent is selected from the group consisting of ethyl acetate, acetonitrile, methanol, ethanol, *n*-propanol, *iso*-propanol, *n*-butanol, *iso*-butanol, acetone, diisopropyl ether, dimethyl formamide, and dimethyl acetamide.

23. (Original) The method of claim 22 wherein the polar solvent is ethyl acetate.

24. (Original) The method of claim 15 wherein the hydrocarbon solvent is selected from the group consisting of *n*-hexane, *n*-heptane, octane, *iso*-octane, cyclohexane, methylcyclohexane, benzene, toluene, and xylene.
25. (Original) The method of claim 24 wherein the hydrocarbon solvent is *n*-hexane.
26. (Original) The method of claim 15 wherein the pH of the water-rich phase is about 8 or higher.
27. (Original) The method of claim 15 wherein the water comprises a base selected from NaOH, KOH, Ca(OH)<sub>2</sub>, NH<sub>3</sub>, Et<sub>3</sub>N, diethylamine and pyridine.
28. (Currently amended) The method of claim 15 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, ~~and~~ everolimus, and ascomycin.
29. (Original) A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:
- a) combining, at a temperature of about 20° to about 25°C, macrolide starting material, ethyl acetate, *n*-hexane, and a water solution of a base selected from NaOH, KOH, Ca(OH)<sub>2</sub>, NH<sub>3</sub>, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N, diethylamine and pyridine whereby at least two phases are formed, one of which is a water-rich phase, wherein the pH of the water-rich phase is > about 7,
  - b) maintaining the combination at a temperature of about 20°C to about 25°C for at least 1 hour, whereby a macrolide-rich phase is formed from which macrolide crystallizes,
  - c) maintaining the combination at a temperature of about 0°C to about 20°C for at least 1 hour, and
  - d) recovering the macrolide that crystallizes.

30. *(Currently amended)* The method of claim 29 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, ~~and~~ everolimus, and ascomycin.

31. *(Original)* The method of claim 29 wherein the pH of the water-rich phase is about 8 or higher.

32. *(Original)* A method of crystallizing a macrolide from a macrolide starting material comprising the steps of:

a) combining, at a temperature of about 20° to about 25°C, a concentrate residue from whole-broth extraction of macrolide-containing biomatter in ethyl acetate, *n*-hexane, and a water solution of a base selected from NaOH, KOH, Ca(OH)<sub>2</sub>, NH<sub>3</sub>, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N, diethylamine and pyridine whereby at least two phases are formed, one of which is a water-rich phase, wherein the pH of the water-rich phase is > about 7,

b) maintaining the combination at a temperature of about 20°C to about 25°C for at least 1 hour, whereby a macrolide-rich phase is formed from which macrolide crystallizes,

c) maintaining the combination at a temperature of about 0°C to about 20°C for at least 1 hour, and

d) recovering the macrolide that crystallizes.

33. *(Currently amended)* The method of claim 32 wherein the macrolide is selected from the group consisting of tacrolimus, sirolimus, pimecrolimus, ~~and~~ everolimus, and ascomycin.

34. *(Original)* The method of claim 32 wherein the pH of the water-rich phase is about 8 or higher.

35. *(Original)* In a method for crystallizing a macrolide from a macrolide starting material, the step of combining the macrolide starting material, a polar solvent, a

hydrocarbon solvent, and water, whereby at least two phases are formed, at least one of which is water rich, wherein the pH of the water-rich phase is at least about 7.

36.     (*Original*) In a method for crystallizing a macrolide from a concentrate residue from whole-broth extraction of macrolide-containing biomatter in a polar solvent, the step of combining the macrolide concentrate in the polar solvent, a hydrocarbon solvent, and water, whereby at least two phases are formed, at least one of which is water rich, wherein the pH of the water-rich phase is at least about 7.